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(54) Title: NATRIURETIC PEPTIDE DERIVATIVES

(57) Abstract

Derivatives of ANP and BNP and analogues thereof having a lipophilic substituent have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides.

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NATRIURETIC PEPTIDE DERIVATIVES

FIELD OF THE INVENTION

The present invention relates to novel derivatives of ANP and BNP, and fragments or extensions thereof and analogues of such fragments and extensions which have a protracted profile of action and to methods of making and using them.

BACKGROUND OF THE INVENTION

Polypeptides are widely used in medical practice, and since they can be produced by recombinant DNA technology it can be expected that their importance will increase also in the years to come. When native polypeptides or analogues thereof are used in therapy it is generally found that they have a high clearance. A high clearance of a therapeutic agent is inconvenient in cases where it is desired to maintain a therapeutically effective blood level thereof over a prolonged period of time since repeated administrations will then be necessary. In some cases it is possible to influence the release profile of polypeptides by applying suitable pharmaceutical compositions, but this approach has various shortcomings and is not generally applicable. The atrial natriuretic (ANP) and brain natriuretic (BNP) peptides are examples of polypeptides with high clearance.. These peptides are characterized by having a central ring structure with a cysteine bridge. Upon metabolization the cysteine bridge and thereby the central core structure is disrupted leaving the peptides inactive. Surprisingly, we found that derivatization of the peptides with a hydrophobic group protected the peptides against metabolic breakdown and thus increased the duration of action considerably.

The amino acid sequence of the peptides are

ANP: Thr-Ala-Pro-Arg-Ser-Leu-Arg-Arg-Ser-Ser-Cys*-Phe-Gly-Gly-Arg-Met-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys*-Asn-Ser-Phe-Arg-Tyr.

BNP: Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys*-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-

Ser-Ser-ser-Gly-Leu-Gly-Cys*-Lys-Val-Leu-Arg-Arg-His.

* indicates that the two cysteines are connected by a disulfide bridge.

SUMMARY OF THE INVENTION

In its broadest aspect, the present invention relates to derivatives of atrial natriuretic peptide or brain natriuretic peptide.

The derivatives according to the invention have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides.

In the present text, the designation "an analogue" is used to designate a peptide wherein one or more amino acid residues of the parent peptide have been substituted by another amino acid residue and/or wherein one or more amino acid residues of the parent peptide have been deleted and/or wherein one or more amino acid residues have been added to the parent peptide.

The term "derivative" is used in the present text to designate a peptide in which one or more of the amino acid residues have been chemically modified, *e.g.* by alkylation, acylation, ester formation or amide formation.

The term "a natriuretic derivative" is used in the present text to designate a derivative of ANP or an analogue thereof or a derivative of BNP or an analogue thereof. In the present text, the parent peptide from which such a derivative is formally derived is in some places referred to as the "natriuretic moiety" of the derivative.

In a preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent attached to any one amino acid residue.

In another preferred embodiment, the present invention relates to a nautriuretic derivative wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25 carbon atoms.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that a carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid residue.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to the parent polypeptide by means of a spacer.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to the parent polypeptide by means of a spacer which is an unbranched alkane α,ω-dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups which spacer forms a bridge between an amino group of the parent polypeptide and an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to the parent polypeptide by means of a spacer which is an amino acid residue except Cys, or a dipeptide such as Gly-Lys.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to the parent polypeptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a carboxyl group of the parent polypeptide forms an amide bond with an amino group of a Lys residue or a dipeptide containing a Lys residue, and the other amino group of the Lys residue or a dipeptide containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to the parent polypeptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein an amino group of the parent polypeptide forms an amide bond with a carboxylic group of the amino acid or dipeptide spacer, and an amino group of the amino acid or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to the parent polypeptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a carboxyl group of the parent polypeptide forms an amide bond with an amino group of the amino acid or dipeptide spacer, and the carboxyl group of the amino acid or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a natriuretic derivative wherein a lipophilic substituent is attached to the parent polypeptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys, and wherein a carboxyl group of the parent polypeptide forms an amide bond with an amino group of Asp or Glu, or a dipeptide containing an Asp or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a straight-chain or branched alkyl group.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is the acyl group of a straight-chain or branched fatty acid.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is an acyl group selected from the group comprising $CH_3(CH_2)_nCO$-, wherein n is 4 to 38, preferably $CH_3(CH_2)_6CO$-, $CH_3(CH_2)_8CO$-, $CH_3(CH_2)_{10}CO$-, $CH_3(CH_2)_{12}CO$-, $CH_3(CH_2)_{14}CO$-, $CH_3(CH_2)_{16}CO$-, $CH_3(CH_2)_{18}CO$-, $CH_3(CH_2)_{20}CO$- and $CH_3(CH_2)_{22}CO$-.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is an acyl group of a straight-chain or branched alkane α,ω-dicarboxylic acid.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is an acyl group selected from the group comprising $HOOC(CH_2)_mCO$-, wherein m is 4 to 38, preferably $HOOC(CH_2)_{14}CO$-, $HOOC(CH_2)_{16}CO$-, $HOOC(CH_2)_{18}CO$-, $HOOC(CH_2)_{20}CO$- and $HOOC(CH_2)_{22}CO$-.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula $CH_3(CH_2)_p((CH_2)_qCOOH)CHNH$-$CO(CH_2)_2CO$-, wherein p and q are integers and p+q is an integer of from 8 to 40, preferably from 12 to 35.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula $CH_3(CH_2)_rCO$-$NHCH(COOH)(CH_2)_2CO$-, wherein r is an integer of from 10 to 24.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula $CH_3(CH_2)_sCO$-$NHCH((CH_2)_2COOH)CO$-, wherein s is an integer of from 8 to 24.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula $COOH(CH_2)_tCO$- wherein t is an integer

of from 8 to 24.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-$CO(CH_2)_uCH_3$, wherein u is an integer of from 8 to 18.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-$COCH((CH_2)_2COOH)NH\text{-}CO(CH_2)_wCH_3$, wherein w is an integer of from 10 to 16.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-$CO(CH_2)_2CH(COOH)NH\text{-}CO(CH_2)_xCH_3$, wherein x is an integer of from 10 to 16.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-$CO(CH_2)_2CH(COOH)NHCO(CH_2)_yCH_3$, wherein y is zero or an integer of from 1 to 22.
In a further preferred embodiment, the present invention relates to a natriuretic derivative which has one lipophilic substituent.

In a further preferred embodiment, the present invention relates to a natriuretic derivative which has two lipophilic substituents.

In a further preferred embodiment, the present invention relates to a natriuretic derivative in which the C-terminal amino acid residue is present in the form of the amide.

In a further preferred embodiment, the present invention relates to a natriuretic derivative having a lipophilic substituent which can be negatively charged.

In a further preferred embodiment, the present invention relates to the use of a natriuretic derivative, wherein the parent polypeptide is selected from the group comprising ANP- (1-28) or

an analogue or a fragment thereof.

In a further preferred embodiment, the present invention relates to the use of an ANP derivative, selected from the group comprising ANP-(2-28), ANP-(3-28), ANP-(4-28), ANP-(5-28) and ANP-(6-28) and an analogue thereof.

In a further preferred embodiment, the present invention relates to the use of an ANP derivative, selected from the group comprising ANP-(1-28)-Pro^{29}-amide and an analogue thereof.

In a further preferred embodiment, the present invention relates to the use of an ANP derivative, wherein the designation analogue comprises derivatives wherein 1-15, preferably 1-10 amino acid residues have been exchanged with any α-amino acid residue, in particular lys.

In a further preferred embodiment, the present invention relates to the use of a natriuretic derivative, wherein the parent polypeptide is selected from the group comprising BNP- (1-32) or an analogue or a fragment thereof.

In a further preferred embodiment, the present invention relates to the use of a BNP derivative, selected from the group comprising BNP-(2-32), BNP-(3-32), BNP-(4-32), BNP-(5-32) , BNP-(6-32), BNP-(7-32), BNP-(8-32) and BNP-(9-32) and an analogue thereof.

In a further preferred embodiment, the present invention relates to the use of a BNP derivative, wherein the designation analogue comprises derivatives wherein 1-15, preferably 1-10 amino acid residues have been exchanged with any α-amino acid residue, in particular lys.

In a further preferred embodiment, the present invention relates to the use of a pharmaceutical composition comprising a natriuretic derivative and a pharmaceutically acceptable vehicle or carrier as described in International Patent Application WO90/01940 (Scios Nova Inc.) and in European Patent Application EP 269299 (Novo Nordisk A/S).

In a further preferred embodiment, the present invention relates to the use of a natriuretic

derivative for the preparation of a medicament with protracted effect.

In a further preferred embodiment, the present invention relates to the use of a natriuretic derivative for the preparation of a medicament with protracted effect for the treatment of hypertension.

In a further preferred embodiment, the present invention relates to the use of a natriuretic derivative for the preparation of a medicament with protracted effect for the treatment of congestive heart failure.

In a further preferred embodiment, the present invention relates to the use of a natriuretic derivative according to for the preparation of a medicament with protracted effect for the treatment of diseases in the renal function.

In a further preferred embodiment, the present invention relates to the use of a natriuretic for the preparation of a medicament with protracted effect for the treatment of oedema.

In a further preferred embodiment, the present invention relates to the use of a natriuretic derivative for the preparation of a medicament with protracted effect for the treatment of hepatic cirrhosis.

DETAILED DESCRIPTION OF THE INVENTION

To obtain a satisfactory protracted profile of action of the natriuretic derivative the lipophilic substituent attached to the natriuretic moiety preferably comprises 4-40 carbon atoms, in particular 8-25 carbon atoms. The lipophilic substituent may be attached to an amino group of the natriuretic moiety by means of a carboxyl group of the lipophilic substituent which forms an amide bond with an amino group of the amino acid to which it is attached. Conversely, the lipophilic substituent may be attached to said amino acid in such a way that an amino group of

the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid. As an alternative, the lipophililic substituent may be linked to the natriuretic moiety via an ester bond. Formally, the ester can be formed either by reaction between a carboxyl group of the natriuretic moiety and a hydroxyl group of the substituent-to-be or by reaction between a hydroxyl group of the natriuretic moiety and a carboxyl group of the substituent-to-be. As a further alternative, the lipophilic substituent can be an alkyl group which is introduced into a primary amino group of the natriuretic moiety.

In one preferred embodiment of the invention, the lipophilic substituent is attached to the natriuretic moiety by means of a spacer in such a way that a carboxyl group of the spacer forms an amide bond with an amino group of the natriuretic moiety. Examples of suitable spacers are succinic acid, Lys, Glu or Asp, or a dipeptide such as Gly-Lys. When the spacer is succinic acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid, and the other carboxyl group thereof may form an amide bond with an amino group of the lipophilic substituent. When the spacer is Lys, Glu or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid, and the amino group thereof may form an amide bond with a carboxyl group of the lipophilic substituent. When Lys is used as the spacer, a further spacer may in some instances be inserted between the ε-amino group of Lys and the lipophilic substituent. In one preferred embodiment, such a further spacer is succinic acid which forms an amide bond with the ε-amino group of Lys and with an amino group present in the lipophilic substituent. In another preferred embodiment such a further spacer is Glu or Asp which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the lipophilic substituent, that is, the lipophilic substituent is a N^{ε}-acylated lysine residue.

In another preferred embodiment of the present invention, the lipophilic substituent has a group which can be negatively charged. One preferred group which can be negatively charged is a carboxylic acid group.

The parent polypeptide can be produced by a method which comprises culturing a host cell containing a DNA sequence encoding the polypeptide and capable of expressing the polypeptide

in a suitable nutrient medium under conditions permitting the expression of the polypeptide, after which the resulting polypeptide is recovered from the culture.

The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (*e.g.* in catalogues of the American Type Culture Collection). The polypeptide produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, *e.g.* ammonium sulphate, purification by a variety of chromatographic procedures, *e.g.* ion exchange chromatography, gelfiltration chromatography, affinity chromatography, or the like, dependent on the type of polypeptide in question.

The DNA sequence encoding the parent polypeptide may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the polypeptide by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (see, for example, Sambrook, J, Fritsch, EF and Maniatis, T, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). The DNA sequence encoding the polypeptide may also be prepared synthetically by established standard methods, *e.g.* the phosphoamidite method described by Beaucage and Caruthers, *Tetrahedron Letters* **22** (1981), 1859 - 1869, or the method described by Matthes *et al.*, *EMBO Journal* **3** (1984), 801 - 805. The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki *et al.*, *Science* **239** (1988), 487 - 491.

The DNA sequence may be inserted into any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, *i.e.* a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.* a plasmid. Alternatively, the vector may be one which, when

introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The vector is preferably an expression vector in which the DNA sequence encoding the polypeptide is operably linked to additional segments required for transcription of the DNA, such as a promoter. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA encoding the polypeptide of the invention in a variety of host cells are well known in the art, cf. for instance Sambrook *et al.., supra.*

The DNA sequence encoding the polypeptide may also, if necessary, be operably connected to a suitable terminator, polyadenylation signals, transcriptional enhancer sequences, and translational enhancer sequences. The recombinant vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question.

The vector may also comprise a selectable marker, *e.g.* a gene the product of which complements a defect in the host cell or one which confers resistance to a drug, *e.g.* ampicillin, kanamycin, tetracyclin, chloramphenicol, neomycin, hygromycin or methotrexate.

To direct a parent polypeptide of the present invention into the secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequence encoding the polypeptide in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide. The secretory signal sequence may be that normally associated with the polypeptide or may be from a gene encoding another secreted protein.

The procedures used to ligate the DNA sequences coding for the present polypeptide, the promoter and optionally the terminator and/or secretory signal sequence, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well

known to persons skilled in the art (cf., for instance, Sambrook *et al.*, *supra*).

The host cell into which the DNA sequence or the recombinant vector is introduced may be any cell which is capable of producing the present polypeptide and includes bacteria, yeast, fungi and higher eukaryotic cells. Examples of suitable host cells well known and used in the art are, without limitation, *E. coli*, *Saccharomyces cerevisiae*, or mammalian BHK or CHO cell lines.

The nautriuretic derivatives can be produced according to the procedures described in the international patent application WO 96/29342.

Compounds which can be useful as natriuretic moieties according to the present invention are described in International Patent Application No. WO 9001940 (Scios Nova Inc.) which relates to ANP analogs which block ANP clearance and are used to treat hypertension, heart disease, renal failure and oedema.

Further natriuretic analogues are described in Euoropean Patent Application EP 269299 (Novo Nordisk A/S) which relates to derivatives of ANP wherein at least two of the amino acid residues in ring structure (Positions 7-23) are exchanged with other amino acid residues and/or which in the C-terminal end (Position 28) are elongated with Pro, Trp, Phe or N-MePhe or a polypeptide moiety having one of the last mentioned four amino acid residues in the N-terminal end, have improved pharmacological properties.

Pharmaceutical compositions

Pharmaceutical compositions containing a polypeptide derivative according to the present invention may be administered parenterally to patients in need of such a treatment. Parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a powder or a liquid for the administration of the polypeptide derivative in the form of a nasal or pulmonal spray. As a still further option, the polypeptide derivatives of the invention can also be

administered transdermally, *e.g.* from a patch, optionally a iontophoretic patch.

Pharmaceutical compositions containing a polypeptide derivative of the present invention may be prepared by conventional techniques, *e.g.* as described in Remington's Pharmaceutical Sciences, 1985.

Thus, the injectable compositions of the polypeptide derivative of the invention can be prepared using the conventional techniques of the pharmaceutical industry which involves dissolving and mixing the ingredients as appropriate to give the desired end product.

Thus, according to one procedure, the polypeptide derivative is dissolved in an amount of water which is somewhat less than the final volume of the composition to be prepared. An isotonic agent, a preservative and a buffer is added as required and the pH value of the solution is adjusted - if necessary - using an acid, *e.g.* hydrochloric acid, or a base, *e.g.* aqueous sodium hydroxide as needed. Finally, the volume of the solution is adjusted with water to give the desired concentration of the ingredients.

Examples of isotonic agents are sodium chloride, mannitol and glycerol.

Examples of preservatives are phenol, m-cresol, methyl p-hydroxybenzoate and benzyl alcohol.

Examples of suitable buffers are sodium acetate and sodium phosphate.

A composition for nasal administration of certain polypeptides may, for example, be prepared as described in European Patent No. 272097 (to Novo Nordisk A/S) or in WO 93/18785.

According to some embodiments of the present invention, the natriuretic derivative is provided in the form of an injectable solution. In such embodiments, the solutions preferably contain not less than about 2 mg/ml, preferably not less than about 5 mg/ml, more preferred not less than about 10 mg/ml of the natriuretic1 derivative and, preferably, not more than about 100 mg/ml of the natriuretic1 derivative.

The polypeptide derivatives of this invention can be used in the treatment of various diseases. The particular polypeptide derivative to be used and the optimal dose level for any patient will depend on the disease to be treated and on a variety of factors including the efficacy of the specific peptide derivative employed, the age, body weight, physical activity, and diet of the patient, on a possible combination with other drugs, and on the severity of the case. It is recommended that the dosage of the polypeptide derivative of this invention be determined for each individual patient by those skilled in the art in a similar way as for known parent polypeptides.

In particular, it is envisaged that the derivatized natriuretic derivative will be useful for the preparation of a medicament with protracted action/effect for the treatment of renal diseases and/or for the treatment of hypertension and/or for the treatment of congestive heart failure.

The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realizing the invention in diverse forms thereof.

CLAIMS

1. A natriuretic derivative comprising a lipophilic substituent attached to any one amino acid residue.

2. A natriuretic derivative according to claim 1, wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25.

3. A natriuretic derivative according to claim 1, wherein said lipophilic substituent is attached to said amino acid in such a way that a carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid.

4. A natriuretic derivative according to claim 1, wherein said lipophilic substituent is attached to said amino acid in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid.

5. A natriuretic derivative according to claim 1, wherein the lipophilic substituent is attached to the parent polypeptide by means of a spacer.

6. A natriuretic derivative according to claim 5, wherein the spacer is an unbranched alkane α,ω-dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups which form a bridge between an amino group of the parent polypeptide and an amino group of the lipophilic substituent.

7. A natriuretic derivative according to claim 5, wherein the spacer is an amino acid residue except Cys, or a dipeptide such as Gly-Lys.

8. A natriuretic derivative according to claim 7, wherein a carboxyl group of the parent polypeptide forms an amide bond with an amino group of Lys or a dipeptide containing a Lys

residue, and the other amino group of the Lys or a dipeptide containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

9. A natriuretic derivative according to claim 7, wherein an amino group of the parent polypeptide forms an amide bond with a carboxylic group of the amino acid or dipeptide spacer, and an amino group of the amino acid or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

10. A natriuretic derivative according to claim 7, wherein a carboxyl group of the parent polypeptide forms an amide bond with an amino group of the amino acid or dipeptide spacer, and the carboxyl group of the amino acid or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

11. A natriuretic derivative according to claim 7, wherein a carboxyl group of the parent polypeptide forms an amide bond with an amino group of Asp or Glu, or a dipeptide containing an Asp or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.

12. A natriuretic derivative according to any of claims 1-4, wherein the lipophilic substituent comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.

13. A natriuretic derivative according to any of claims 1-4, wherein the lipophilic substituent is an straight-chain or branched alkyl group.

14. A natriuretic derivative according to any of claims 1-5, 8 and 9 wherein the lipophilic substituent is the acyl group of a straight-chain or branched fatty acid.

15. A natriuretic derivative according to claim 14 wherein the acyl group is selected from the group comprising $CH_3(CH_2)_nCO$-, wherein n is 4 to 38, preferably $CH_3(CH_2)_6CO$-, $CH_3(CH_2)_8CO$-, $CH_3(CH_2)_{10}CO$-, $CH_3(CH_2)_{12}CO$-, $CH_3(CH_2)_{14}CO$-, $CH_3(CH_2)_{16}CO$-, $CH_3(CH_2)_{18}CO$-, $CH_3(CH_2)_{20}CO$- and $CH_3(CH_2)_{22}CO$-.

16.A natriuretic derivative according to any of claims 1-5, 8 and 9 wherein the lipophilic substituent is an acyl group of a straight-chain or branched alkane α,ω-dicarboxylic acid.

17.A natriuretic derivative according to claim 16 wherein the acyl group is selected from the group comprising $HOOC(CH_2)_mCO$-, wherein m is 4 to 38, preferably $HOOC(CH_2)_{14}CO$-, $HOOC(CH_2)_{16}CO$-, $HOOC(CH_2)_{18}CO$-, $HOOC(CH_2)_{20}CO$- and $HOOC(CH_2)_{22}CO$-.

18.A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophilic substituent is a group of the formula $CH_3(CH_2)_p((CH_2)_qCOOH)CHNH\text{-}CO(CH_2)_2CO$-, wherein p and q are integers and p+q is an integer of from 8 to 40, preferably from 12 to 35.

19.A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophlic substituent is a group of the formula $CH_3(CH_2)_rCO\text{-}NHCH(COOH)(CH_2)_2CO$-, wherein r is an integer of from 10 to 24.

20.A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophilic substituent is a group of the formula $CH_3(CH_2)_sCO\text{-}NHCH((CH_2)_2COOH)CO$-, wherein s is an integer of from 8 to 24.

21.A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophilic substituent is a group of the formula $COOH(CH_2)_tCO$- wherein t is an integer of from 8 to 24.

22.A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophilic substituent is a group of the formula $\text{-}NHCH(COOH)(CH_2)_4NH\text{-}CO(CH_2)_uCH_3$, wherein u is an integer of from 8 to 18.

23.A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophilic substituent is a group of the formula $\text{-}NHCH(COOH)(CH_2)_4NH\text{-}COCH((CH_2)_2COOH)NH$-

$CO(CH_2)_wCH_3$, wherein w is an integer of from 10 to 16.

24. A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophilic substituent is a group of the formula $-NHCH(COOH)(CH_2)_4NH-CO(CH_2)_2CH(COOH)NH-CO(CH_2)_xCH_3$, wherein x is an integer of from 10 to 16.

25. A natriuretic derivative according to any of claims 1-5, 8 and 9, wherein the lipophilic substituent is a group of the formula $-NHCH(COOH)(CH_2)_4NH-CO(CH_2)_2CH(COOH)NHCO(CH_2)_yCH_3$, wherein y is zero or an integer of from 1 to 22.

26. A natriuretic derivative according to any of the preceding claims which has one lipophilic substituent.

27. A natriuretic derivative according to any one of claims 1-25 which has two lipophilic substituents.

28. A natriuretic derivative according to any of claims 1-27, wherein the parent polypeptide is selected from the group comprising ANP-(1-28) or an analogue or a fragment thereof.

29. An ANP derivative according to claim 28, selected from the group comprising ANP-(2-28), ANP-(3-28), ANP-(4-28), ANP-(5-28) and ANP-(6-28) and an analogue thereof.

30. An ANP derivative according to claim 1-27, selected from the group comprising ANP-(1-28)-Pro^{29}-amide and an analogue thereof.

31. An ANP derivative according to any of the claims 28-30 wherein 1-15, preferably 1-10 amino acid residues have been exchanged with any α-amino acid residue, in particular lys.

32. A natriuretic derivative according to any of claims 1-27, wherein the parent polypeptide is selected from the group comprising BNP-(1-32) or an analogue or a fragment thereof.

33.A BNP derivative according to claim 32, selected from the group comprising BNP-(2-32), BNP-(3-32), BNP-(4-32), BNP-(5-32) , BNP-(6-32), BNP-(7-32), BNP-(8-32) and BNP-(9-32) and an analogue thereof.

34.A BNP derivative according to any of the claims 32-33 wherein 1-15, preferably 1-10 amino acid residues have been exchanged with any α-amino acid residue, in particular lys.

35.A pharmaceutical composition comprising a natriuretic derivative according to any of the preceding claims and a pharmaceutically acceptable vehicle or carrier.

36.Use of a natriuretic derivative according to any of the preceding claims for the preparation of a medicament with protracted effect.

37.Use of a natriuretic derivative according to any of claims 28-34 for the preparation of a medicament with protracted effect for the treatment of hypertension.

38.Use of a natriuretic derivative according to any of claims 28-34 for the preparation of a medicament with protracted effect for the treatment of congestive heart failure.

39.Use of a natriuretic derivative according to any of claims 28-34 for the preparation of a medicament with protracted effect for the treatment of diseases in the renal function.

40.Use of a natriuretic derivative according to any of claims 28-34 for the preparation of a medicament with protracted effect for the treatment of oedema.

41.Use of a natriuretic derivative according to any of claims 28-34 for the preparation of a medicament with protracted effect for the treatment of hepatic cirrhosis.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 98/00142

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/58, A61K 38/22
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, PAJ, MEDLINE, EMBASE, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 8702674 A1 (BIOTECHNOLOGY RESEARCH ASSOCIATES, J.V.), 7 May 1987 (07.05.87), whole document, especially pages 6-9	1-41
X	WO 8909611 A1 (CALIFORNIA BIOTECHNOLOGY, INC.), 19 October 1989 (19.10.89), whole document, especially"Disclosure of invention"and page 27, line 29 - page 28, line 12	1-41
X	WO 9513296 A1 (GENENTECH, INC.), 18 May 1995 (18.05.95), "Summary of invention"and page 25, line 14-26	1-41

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Information on patent family members

30/06/98

International application No.
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